IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	: 10/549,685	Confirmation No.	7434
Applicant	: Eva CAROFF, *et al.*		
Filed	: September 19, 2005		
Title	: **GUANIDINE DERIVATIVES AND THEIR USE AS NEUROPEPTIDE FF RECEPTOR ANTAGONISTS**		
	:		
TC/Art Unit	1617		
Examiner:	Manu M. MANOHAR		
Docket No.	: 66535.000004		
Customer No.	: **21967**		

MAIL STOP AMENDMENT

Commissioner for Patents

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Declaration of Dr. Markus A. Riederer

I, Dr. Markus.A. Riederer, hereby declare that:

1. I have worked for Axovan Pharmaceuticals Ltd. from Sept. 2002 until November 2003. In November 2003 Actelion Pharmaceuticals Ltd. purchased Axovan Pharmaceuticals Ltd. I have worked for Actelion Pharmaceuticals Ltd. from Nov. 2003 until the present. My current title is Deputy Head Drug Discovery Biology, Senior Group Leader.

2. I was awarded my Master degree in 1987 from University of Basel, Switzerland, in Cell Biology, and my Ph.D. in 1991 from University of Basel, Switzerland in Biochemistry. My doctoral studies concerned "Influence of N-linked Glycosylation on the Efficiency of Secretion of Yeast Acid Phosphatase".

3. During my employments at Axovan Pharmaceuticals Ltd. and at Actelion Pharmaceuticals Ltd., I have worked on NPFF. Currently I am a Senior Group Leader and have been in this position for the last 5 years.

4. I have read and understand the specification of United States Patent Application No. 10/549,685, published on August 31, 2006 as U.S. Patent Application Publication No. 2006/0194788.

5. The specification includes tests of certain compounds of Formula I for their affinity to the NPFF receptors.

6. The specification describes the tests as follows:

Hamster cells suitable for Neuropeptide FF receptor-binding studies (Chinese Hamster Ovary cells, CHOSP10) which in each case produce the NPFF1 or NPFF2 receptor, were multiplied in standard cell-culture conditions. The cell-culture medium was sucked out and 5 ml of buffer A (5 mM Tris pH=7.4, 1 mM $MgCl_2$) added per 17 cm Petri dish. The cells were scraped off the cell-culture plate and transferred into a 50 ml Falcon vessel. The cells were then centrifuged for 5 minutes at 450 g, resuspended in buffer A once again and mixed for 30 seconds on a Polytron vortex. After centrifugation at 30,000 g for 20 minutes the supernatant was discarded and the membrane pellet taken up in 500 µl buffer C (75 mM Tris pH=7.4, 25 mM $MgCl_2$, 250 mM sucrose, 0.1 mM PMSF, 0.1 mM phenanthroline). The membrane-buffer mixture was then divided into aliquots and deep-frozen. The protein content of an aliquot was determined by the Lowry method.

The binding test was carried out in a final volume of 250 µl 100 µl membrane-buffer mixture corresponding to 35 µg protein content was mixed with 95 µl binding buffer (50

mM Tris pH 7.4, 60 mM NaCl, 0.1% protease-free BSA, 0.01% NaN_3). After addition of 5 µl each of a concentration of test substance per measurement point, 0.2 nM ^{125}I-Tyr1-NPFF (NEN, NEX381) per measurement point was added in 50 µl. After 90 minutes incubation at room temperature the samples were sucked out through a GF/C filter (Millipore (MAHFC1H60)) and the filter was washed with ice cold binding buffer with 3 times 300 µl (Packard Filtermate). After addition of 55 µl Microscint 40 (Packard 6013641) scintillation fluid the measurement points were quantified in the gamma counter (Packard, Top Count NXT).

Non-specific binding was ascertained in the presence of 1 µM unmarked neuropeptide FF. Specific binding is defined as the difference between total and non-specific binding.

IC_{50} values are defined as that concentration of the antagonist which displaces 50% of the ^{125}-I-marked neuropeptide FF. This concentration is ascertained by linear regression analysis after logit/log-transformation of the binding values.

7. The specification includes the following IC_{50} values:

TABLE 1

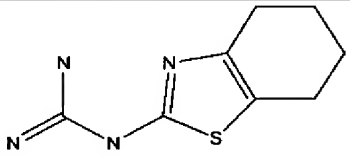
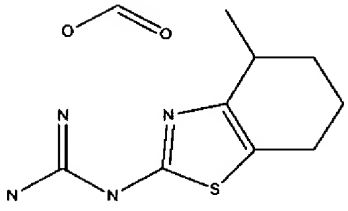
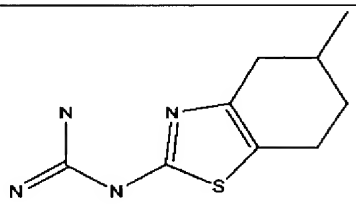
NPFF1 receptor binding

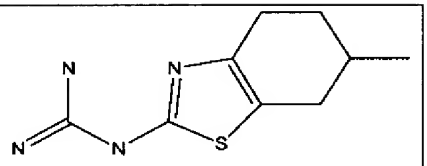
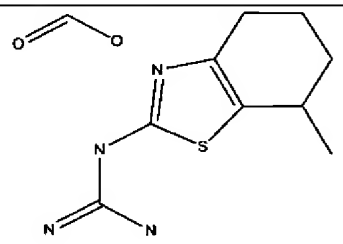
Compound	Binding NPFF-1 IC50 [□M]
N-(5-ethyl-5-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.0002
N-(5,5-dimethyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.002
N-(4-tert-butyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.002
N-(5,5-dimethyl-6-phenyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.002
N-(6-isopropyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.004
N-(6,6-dimethyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.004
N-(5,5,7-trimethyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.004
N-(5-butyl-5,6,7,8-tetrahydro-4H-cycloheptathiazol-2-yl)-guanidine	0.005
N-(5-butyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.005
N-(4-ethyl-4-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.005
N-[6-(3,4-dimethoxyphenyl)-4,5,6,7-tetrahydro-benzothiazole-2-yl]-guanidine	0.005
N-(5-Methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.006
N-(6-phenyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.006
N-(6-propyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.007
N-(4-methyl-4-propyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.007
N-(4-cyclohex-1-enyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.008
N-(4-sec-butyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.009
N-(4-isobutyl-4-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.009
N-(6-tert-butyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	0.010

8. The specification does not provide comparative data for N-(4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine and the claimed methyl substituted compounds: N-(4-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine formate; N-(5-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine; N-(6-methyl 4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine; N-(7-methyl-4,5,6,7-tetrahydro-benzothiazol-2-yl)-guanidine formate.

9. The methyl substituted compounds listed in the preceding paragraph were tested for their affinity to the NPFF receptors and the results are provided in the table below:

Table 2

Example No	Name	IC_{50} [nM]	Structure
C-15	N-(4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	210	
C-25	N-(4-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine formate	40	
C-02	N-(5-methyl-4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	6	

C-10	N-(6-methyl 4,5,6,7-tetrahydro-benzothiazole-2-yl)-guanidine	62	
C-71	N-(7-methyl-4,5,6,7-tetrahydro-benzothiazol-2-yl)- guanidine formate	12	

10. As shown in the table above, the methyl-substituted compounds C-25, C-02, C-10, and C-71 have significantly lower IC_{50} values than the unsubstituted C-15 compound.

11. To the extent that a person having ordinary skill in the art would have expected that methyl substitution would have yielded a compound having similar properties, including a similar IC_{50} value, the results in Table 2 above demonstrate that methyl substitution have significantly higher NPFF activity and correspondingly lower IC_{50} values.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

M. Riederer Sept 2nd 2009

Dr. Markus A. Riederer Date